

# Multi-cancer detection of early-stage cancers with simultaneous tissue localization using a plasma cfDNA-based targeted methylation assay

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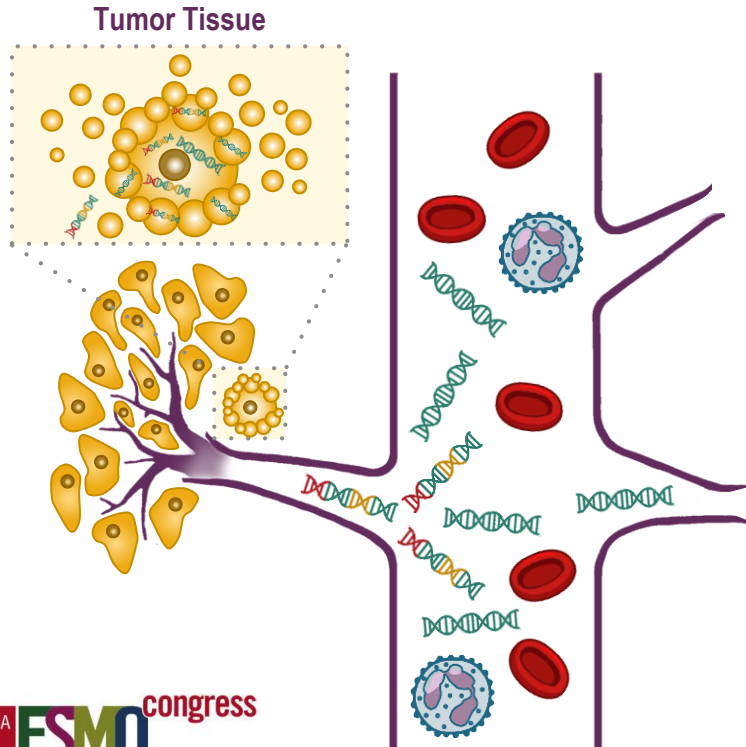
# DISCLOSURE INFORMATION

Geoffrey R. Oxnard, MD

- Consulting fees from AstraZeneca; Sysmex; Takeda; Boehringer-Ingelheim; Inivata; Genentech; Loxo; DropWorks; GRAIL, Inc.; Janssen; Illumina.
- Honoraria from Chugai, BioRad, Guardant, Foundation Medicine.
- Licensing fees from MolecularMD paid to my institution.
- Institutional clinical trial support from AstraZeneca; Loxo; Lilly; Boehringer-Ingelheim; Pfizer; Astellas; GRAIL, Inc.
- Chair, Scientific Leadership Board, GO2 Lung Cancer Foundation.

# Cancer is a Disease of the Genome

Tumors shed cell-free DNA into the blood, carrying signals specific to cancer



## Hallmarks of cancer DNA in the blood:



Mutations  
(Single Base Changes)



Chromosome Alterations  
(Copy Number)



DNA Methylation Patterns  
(Chemical Modification)

# Requirements for Multi-cancer Test for Use at Population Scale

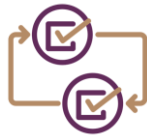
## Benefits of early detection while minimizing harms:

- **Low false positive rate:** achieved through high specificity
- **Localizing ability:** ability to identify anatomic location to direct appropriate diagnostic work-up
- **Limited over-diagnosis:** preferential detection of clinically significant cancers

## Demonstrate test performance, reproducibility, and generalizability to population:



Pre-specified statistical analyses to reduce bias



Assessment of performance in an independent test set



Inclusion of potentially confounding conditions to ensure specificity



Multiple study sites for demographic diversity



Evaluation of performance in population scale studies with people with no known diagnosis

# The Circulating Cell-free Genome Atlas (CCGA) Study: Supporting Development of a Multi-Cancer Test

Prospective, observational, longitudinal, case-control study for discovery, training, and validation of multi-cancer test



15,254 participants  
with & without cancer

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142 sites

**Fully Enrolled**



**Blood samples**  
(from all participants)



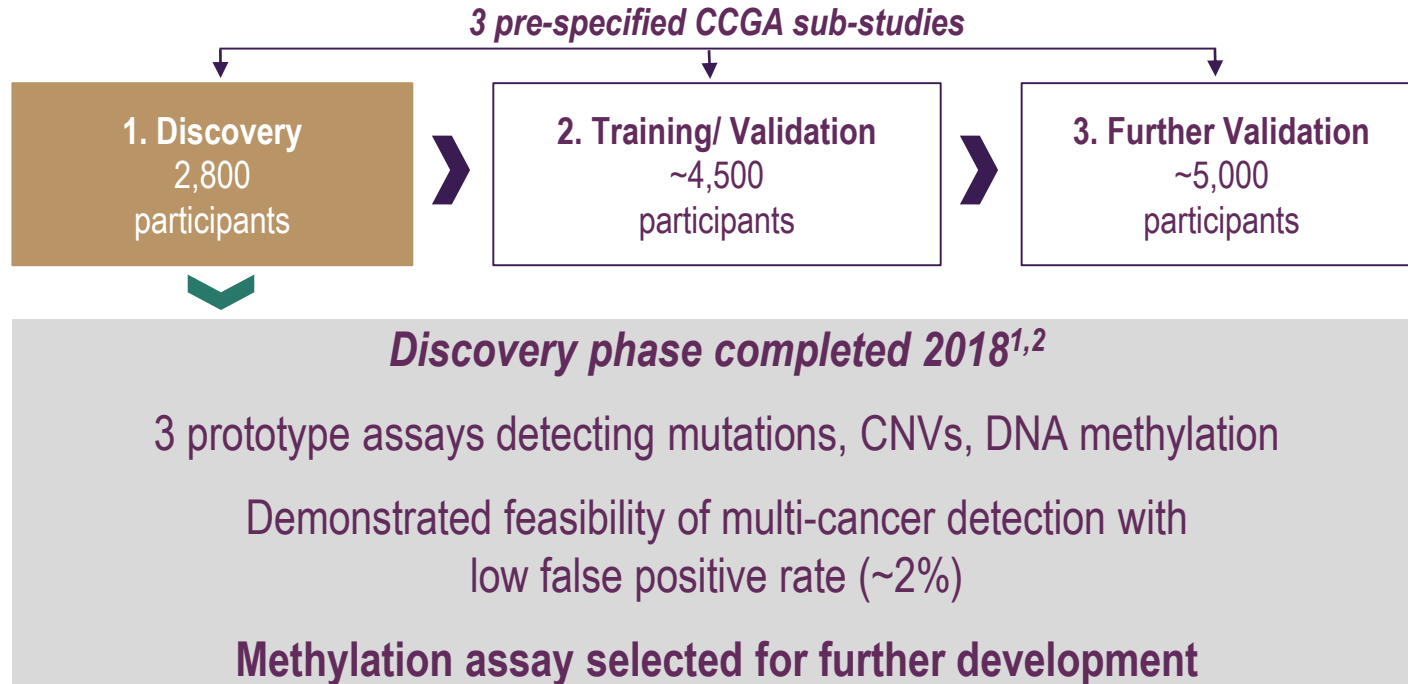
**Tissue samples**  
(cancer only)



**Follow-up for 5 years**  
(vital status & cancer status)

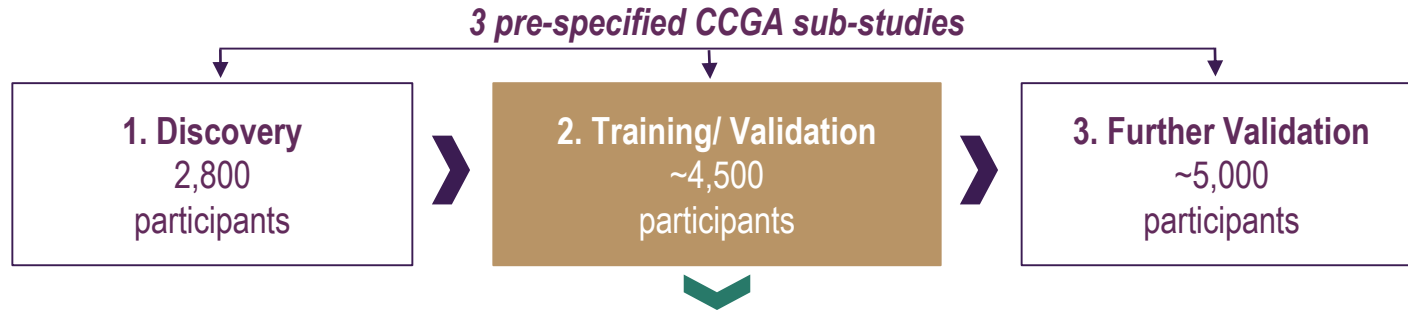
# The CCGA Study: Targeted Methylation Assay for Further Development

## Pre-specified studies for discovery and validation



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Pre-specified studies for discovery and validation



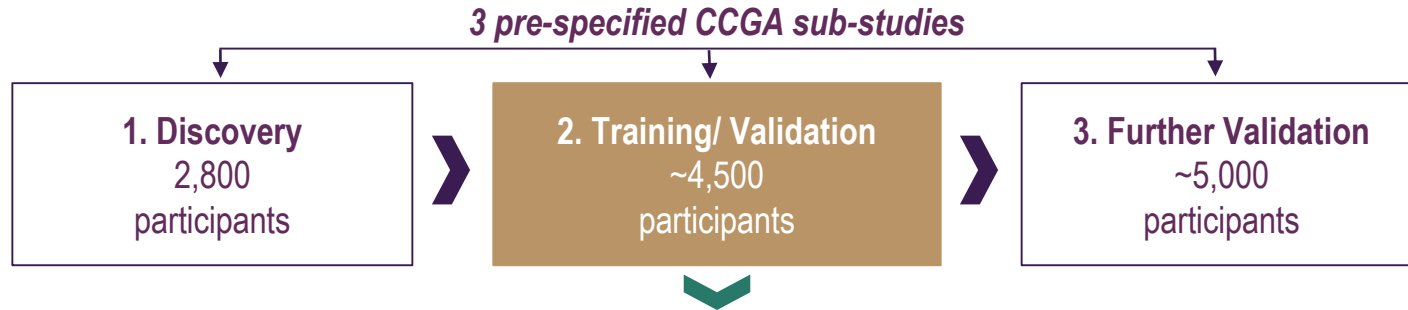
A **targeted methylation assay** was developed targeting key informative genomic regions

Here, we report training and internal cross-validation

Assessment of an independent validation set is ongoing

# The CCGA Study: Targeted Methylation Assay for Further Development

## Pre-specified studies for discovery and validation



### 14 pre-specified cancers types:

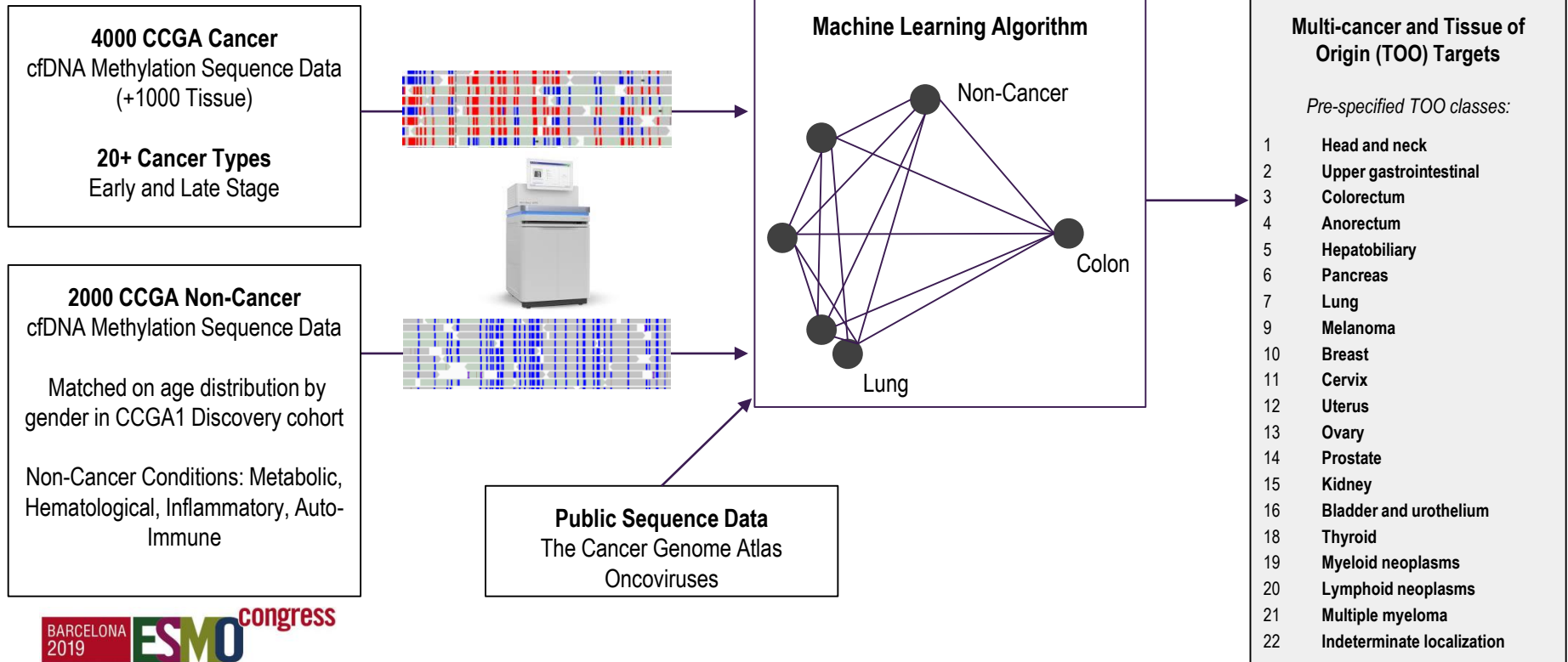
Anorectal, hormone receptor-negative breast, colorectal, esophagus, gallbladder, gastric, head and neck, hepatobiliary, lung, lymphoid neoplasm, lymphoma, multiple myeloma, ovary, pancreas

**These cancer types account for ~63% of US cancer deaths<sup>1</sup>**



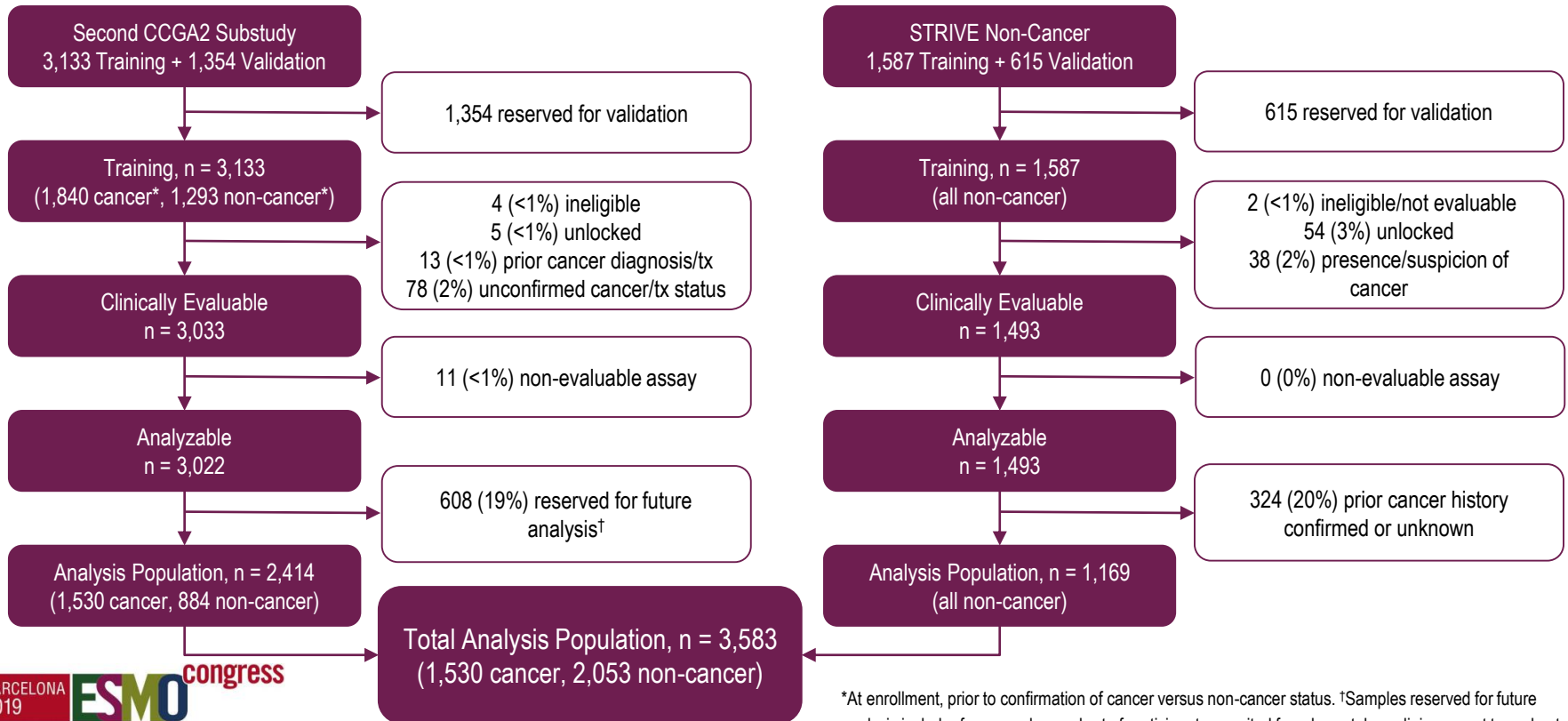
# Target Selection using Machine Learning Algorithm

Targeted methylation panel developed through generation and analysis of an extensive database of plasma and tissue methylation patterns



# Cancer and Non-Cancer Participant Disposition

To reach >99% specificity with >90% confidence, additional non-cancer samples were incorporated from an independent observational cohort study



\*At enrollment, prior to confirmation of cancer versus non-cancer status. †Samples reserved for future analysis include, for example, a cohort of participants recruited from hematology clinics meant to understand cfDNA signal in premalignant or other hematologic conditions.

# Comparable Cancer and Non-Cancer Groups

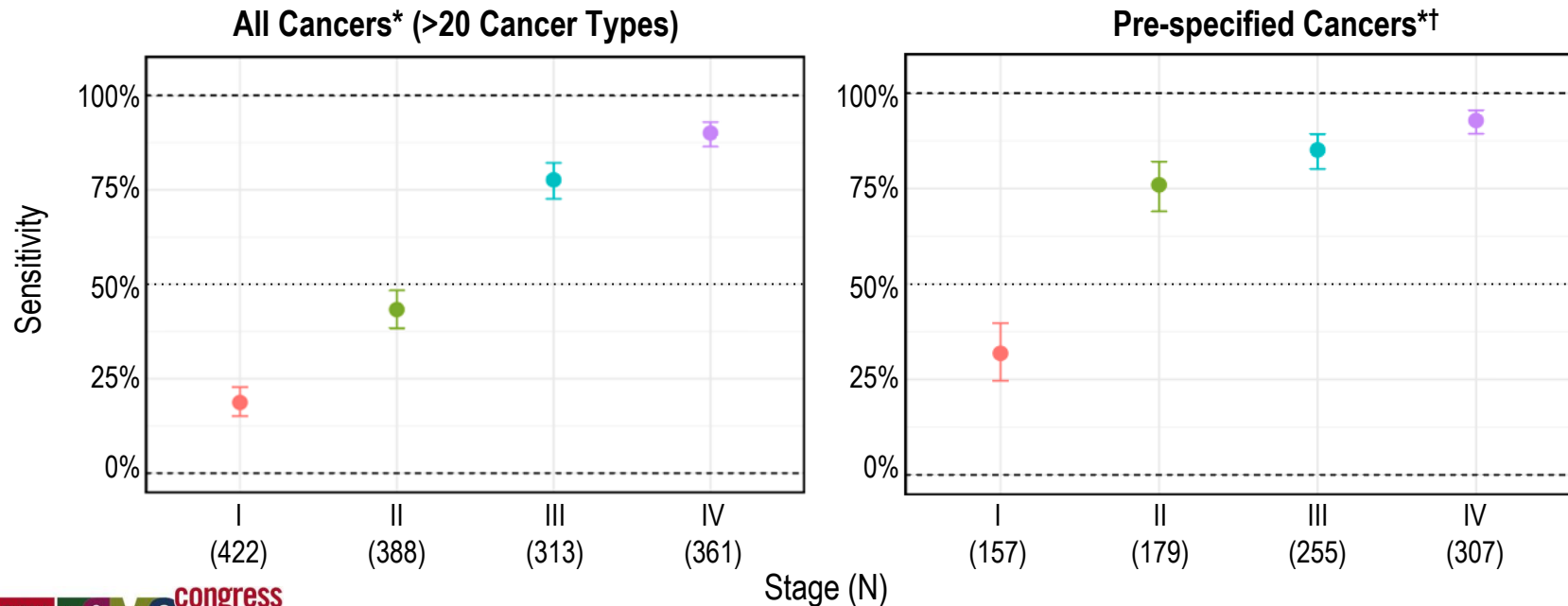
	Second CCGA Substudy		STRIVE
	Cancer N = 1,530	Non-Cancer N = 884	Non-Cancer N = 1,160
Total (N=3,133)			
Age, Mean $\pm$ SD	62.1 $\pm$ 12.0	54.3 $\pm$ 13.6	60.6 $\pm$ 9.6
Female, N (%)	763 (49.9%)	585 (66.2%)	1,169 (100%)
Race/Ethnicity, N (%)			
White, Non-Hispanic	1,263 (83.1%)	719 (81.4%)	1,017 (87.7%)
Black, Non-Hispanic	105 (6.9%)	66 (7.5%)	7 (<1%)
Hispanic, Asian, Other	152 (10.0%)	98 (11.1%)	136 (11.7%)
Never-smoker, N (%)	679 (45.2%)	500 (57.3)	716 (62.5%)
BMI, Normal/Underweight, N (%)	415 (27.1%)	218 (24.7%)	493 (42.2%)

# Broad Cancer Stage Distribution

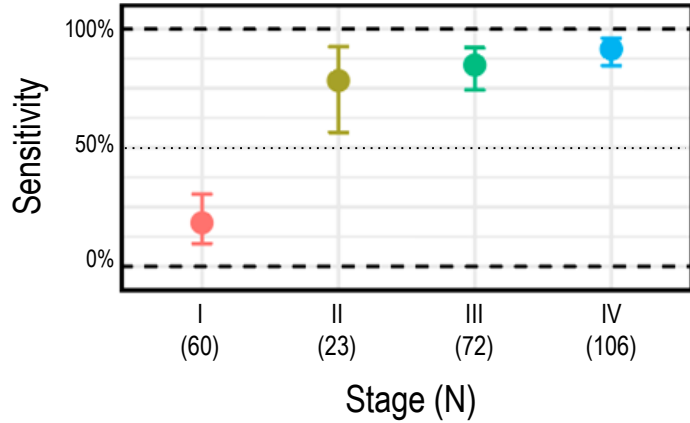
	Second CCGA Substudy		STRIVE
	Cancer N = 1,530	Non-Cancer N = 884	Non-Cancer N = 1,160
Clinical Staging, N (%)			
Stage I	422 (27.6%)	--	--
Stage II	388 (25.4%)	--	--
Stage III	313 (20.5%)	--	--
Stage IV	361 (23.6%)	--	--
Unstaged*	46 (3.0%)	--	--
Method of Diagnosis, N (%)			
Screening	367 (24.0%)	--	--

# Cancers Detected at Early and Late Stages at 99.4% Specificity

- 54.7% (95% CI: 52.2-57.2%) overall sensitivity (>20 cancer types)
- 75.8% (72.9-78.5%) sensitivity in pre-specified† cancer types
- Single fixed false-positive rate (99.4% specificity) across >20 cancer types

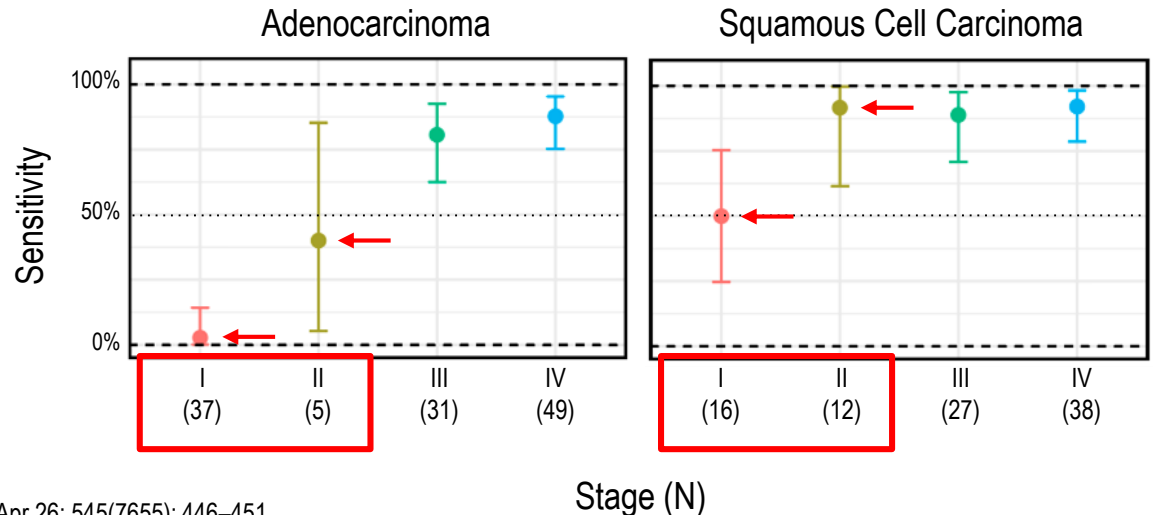


# Lung Cancer Detection Varies by Subtype at 99.4% Specificity

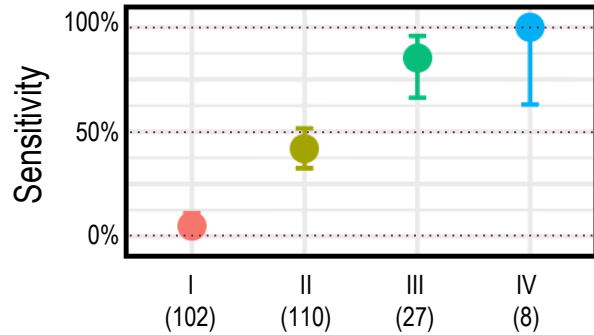


- Detection rate affected by early-stage adenocarcinomas
  - Detection higher in squamous cell carcinoma
- Consistent with prior report showing ctDNA detection was higher in squamous cell carcinoma than adenocarcinoma<sup>1</sup>

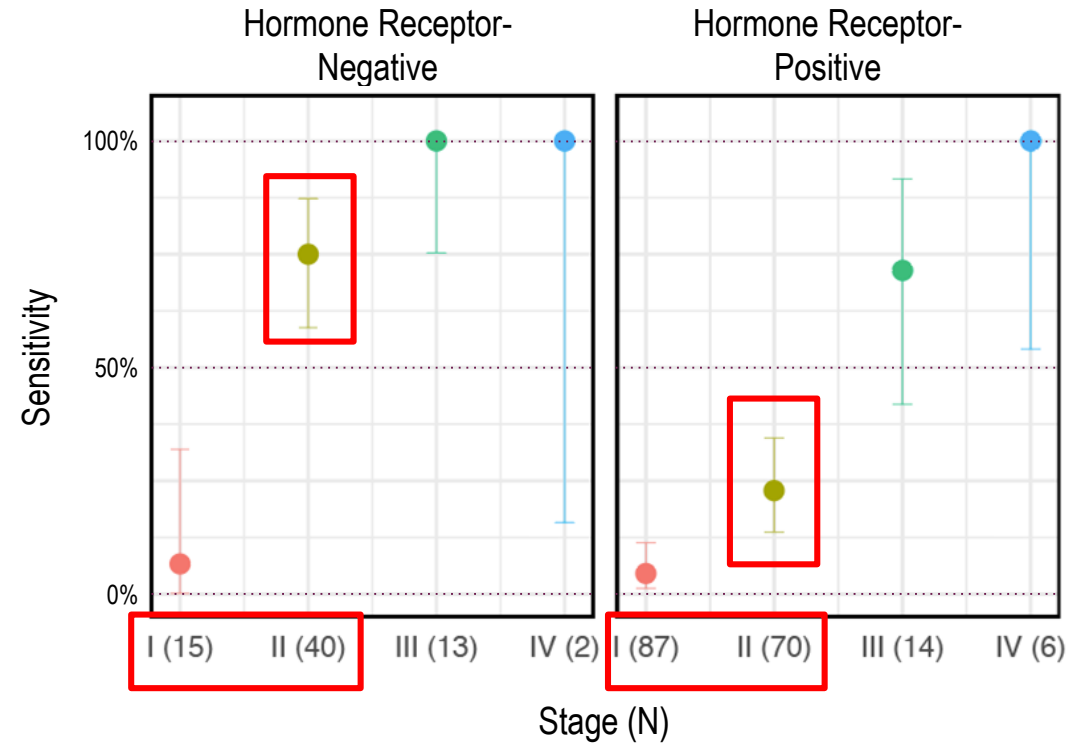
- Overall lung cancer sensitivity: 71.6% (95% CI: 65.8-77.0%)



# Breast Cancer Detection Varies by Subtype at 99.4% Specificity

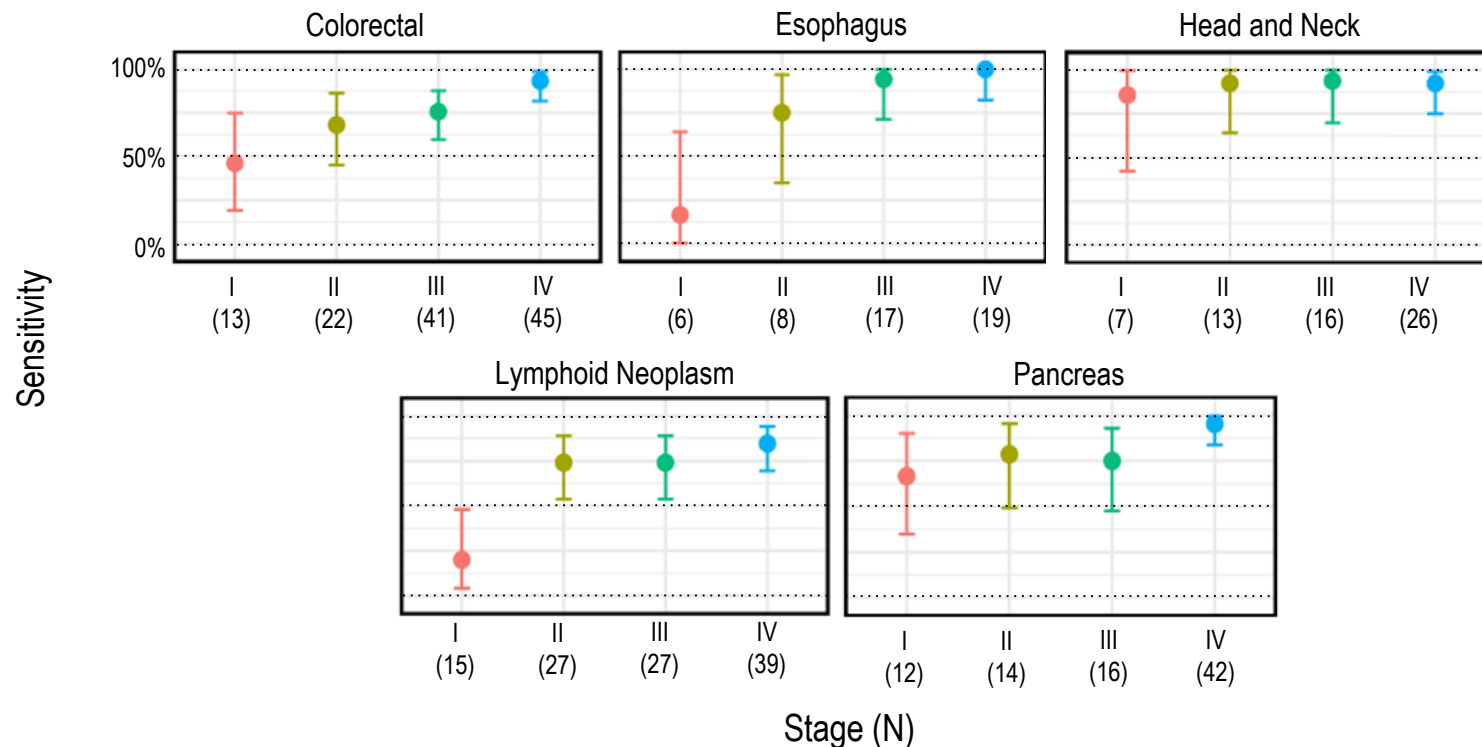


- Overall breast cancer sensitivity: 33.2% (95% CI: 27.4-39.4%)
- Detection rate affected by preponderance of participants with early-stage hormone receptor-positive breast cancer



# Additional Pre-Specified Cancer Detection\* at 99.4% Specificity

Many cancer types lack screening paradigms



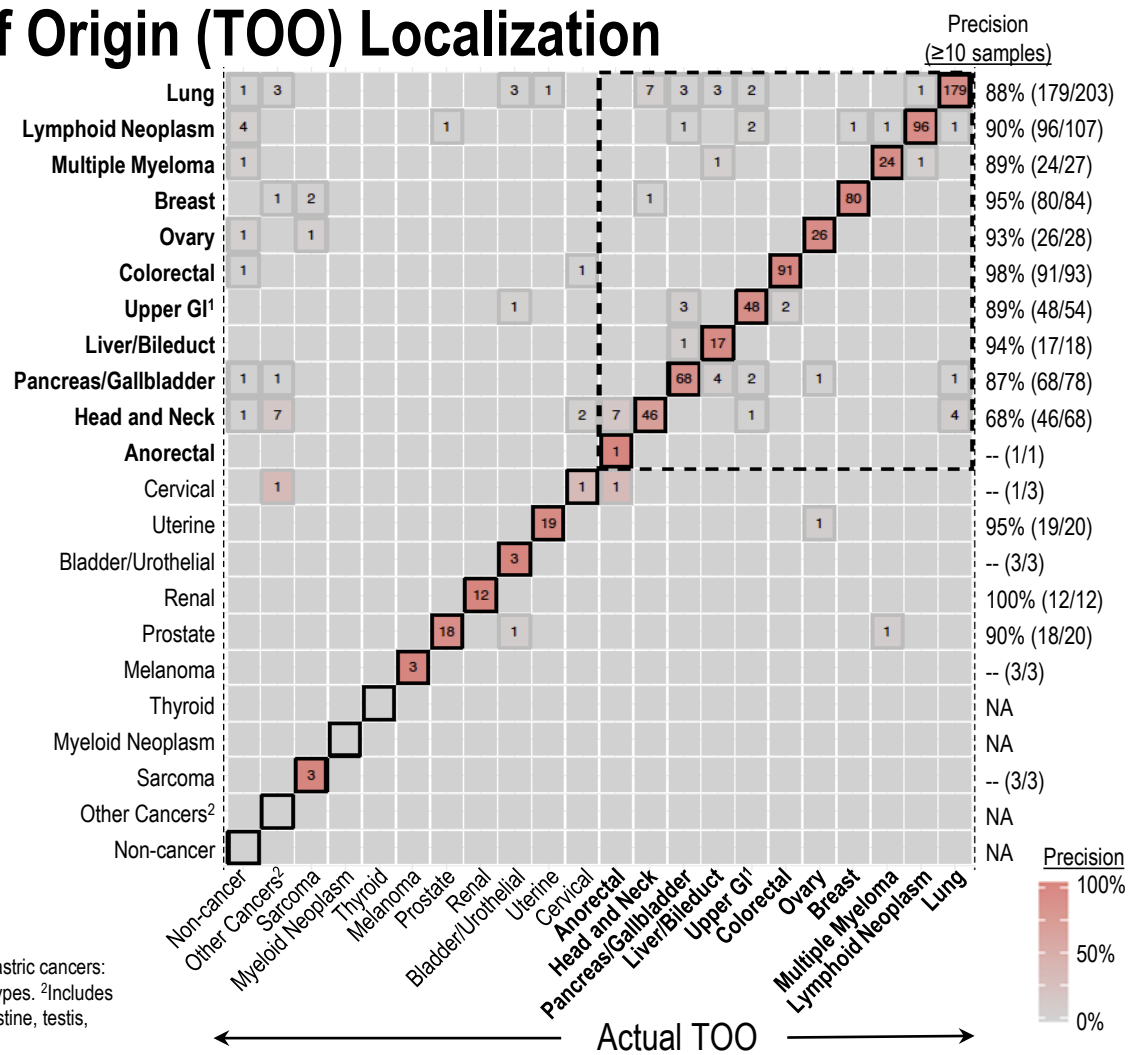
\*Cancer types with >50 samples displayed (not displayed: anorectal, hormone receptor-negative breast, gallbladder, gastric, hepatobiliary, leukemia, lung, multiple myeloma, ovary).



# Highly Accurate Tissue of Origin (TOO) Localization

- 97% of samples with assigned TOO
- 89% of those calls were correct
- Highly precise localization to a single tissue site across >20 distinct tumor types

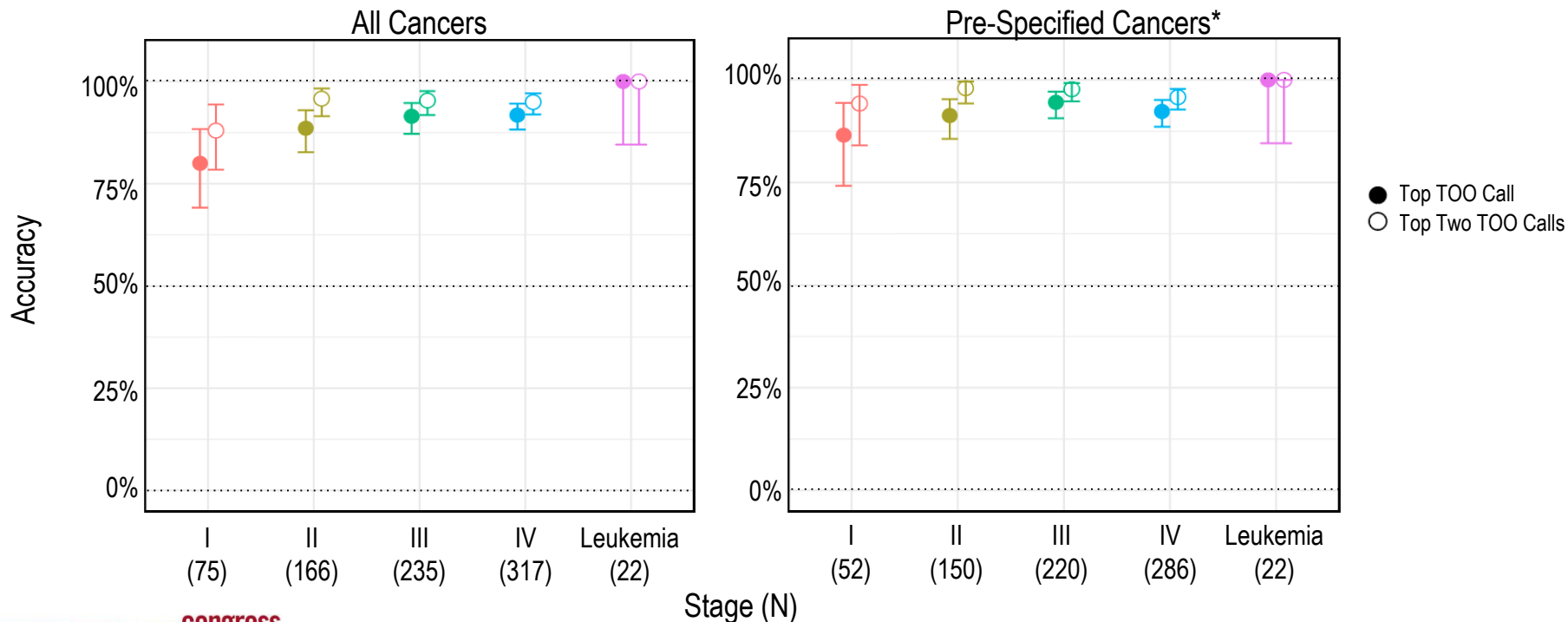
Predicted TOO



<sup>1</sup>Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types. <sup>2</sup>Includes mesothelioma, penis, pleura, small intestine, testis, unspecified, vulva.

# Tissue of Origin (TOO) Accuracy is Consistently High Across Stages

- Single tissue localization: **89%** (~9 of 10) of TOO calls were correct
- Localization to the top two TOO calls: **94%** (~19 of 20) of TOO calls were correct



# Conclusions

- Targeted methylation analysis of cfDNA simultaneously detected multiple cancer types, at early stages, at a specificity (>99%) appropriate for population screening
  - Detection of >20 cancer types was achieved with a **single, fixed, low false positive rate**
  - This approach also **accurately localized the TOO**, which will streamline subsequent diagnostic work-up
  - Both should be requirements for a blood-based multi-cancer test
- Results from an independent validation set will be presented at a future meeting
- Together, **these findings support the further clinical development of this targeted methylation approach as a multi-cancer detection test for numerous clinically significant cancer types**

# Acknowledgements

- Study participants who graciously donated their time, energy, and specimens
- Investigators and collaborators for advice, enrolling participants, and collecting data and specimens
- Advisors and Scientific Advisory Board members for their helpful feedback and advice
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